DELAY IN IMPLANTATION IN LACTATING RATS IN RELATION TO ENERGY CONSUMPTION*

NIVA SHAPIRA, J. KALI, S. AMIR AND H. SCHINDLER

*The Volcani Center, Agricultural Research Organization,
Bet Dagan, Israel*

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Summary. Rats mated at the *post-partum* oestrus, each of them nursing ten young, were allocated to three feeding groups, one maintained on 62% and another on 41% of the daily amounts consumed by the third group, which was fed freely. The composition of the food mixtures for the three groups was adjusted so that the lower consumption was at the expense of grains only.

Energy restrictions inhibited implantation at a high incidence until the 18th day of lactation, when the restriction was lifted. Energy restriction also caused lower delivery rates. This was assumed to be due to unaccomplished implantation after interruption of the blastocystic diapause.

The young nursed by the dams subjected to food restriction were retarded. It was suggested that the energy deficit resulting from heavy lactation was responsible for the extended delay in implantation by depressing gonadotrophic function and by increasing suckling intensity in the retarded young.

INTRODUCTION

An ordinary implantation delay of 1 to 7 days was found in 90% of lactating rats, but in a further 5% of such rats the delay was extended up to 8 to 16 days (Mantalenakis & Ketchel, 1966). There is good agreement that the delay of implantation during lactation is a result of depressed gonadotrophic function caused by the suckling stimulus (McCann & Ramirez, 1964; McCann, 1965; Psychoyos, 1967). Undernutrition also caused gonadotrophic hypofunction (Werner, 1939; Mulinos & Pomerantz, 1940; Meites & Reed, 1949; Leatham, 1966), and the effect of inanition on fertility was described as 'pseudohypophysectomy' (Pomerantz & Mulinos, 1939). Dietary restriction (Lee, King & Visscher, 1952) and low protein level (Nelson & Evans, 1953) were found to affect certain reproductive functions in female rats.

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The present paper reports the effect of energy restriction on time and rate of implantation in rats.

MATERIALS AND METHODS

Ninety-three parturient rats, 4 months old, were mated at the *post-partum* oestrus. These rats were allocated at Day 1 (Day 0, the day on which a vaginal plug was found) into three feeding groups: a control group which was fed freely, and two groups with restricted food consumption. The food restriction was planned to supply 75% and 50% of the daily 'free consumptions', which were determined in a preliminary study on similar rats. Each of the females of the experiment, as well as those of the preliminary study, were given ten young.

The food mixtures contained the following ingredients for the 'free-fed', '75%' and '50%' groups, respectively: corn meal: 58.5, 44.7, 17.0%; soybean meal: 21.4, 28.5, 42.8%; skim milk powder: 15.6, 20.8, 31.2%; soybean oil: 2.2, 2.9, 4.4%; dicalcium phosphate: 1.0, 1.3, 2.0%; table salt: 1.0, 1.3, 2.0%; commercial vitamin mixture: 0.3, 0.4, 0.6%. The protein content of the three mixtures was 31, 24 and 19%, respectively.

Food restriction was imposed on the females from the morning of Day 1 until Day 18 of lactation. After this day, all females were fed freely because, at that time, the young shared their mothers' food, thus making an accurate determination of the females' food consumption impossible. Since ovulation occurs at about 02.00 hours after delivery, the feeding schedule ensured that food restriction was not imposed earlier than 40 hr after ovulation and avoided any adverse effect on ovulation, fertilization and egg transport.

The numbers of parturient and non-parturient females and the length of pregnancy were determined in rats that were maintained to term. For studying the pre- and post-implantation losses, females of each group were killed on Days 5, 12 and 18. No further immediate effects of food restriction were expected after Day 18. The rats were inspected for the presence of implantation sites and, when these were absent, the uterine horns were flushed with saline and the presence or absence of free blastocysts in the washings was determined.

The actual food consumption was checked every other day, and the young were weighed at the same intervals.

RESULTS

The rats of the control group consumed more food than those in the preliminary study, and therefore the rats in the restricted groups consumed 62% and 41% of the 'free consumption' instead of the planned levels of 75% and 50% (Text-fig. 1).

Fewer females produced litters among the restricted groups than among the free-fed group (Table 1). The females of the free-fed group gave birth after expected pregnancy periods (Mantalenakis & Ketchel, 1966) of 24 to 26 days. This fact implies that implantation occurred around Day 9 of pregnancy-lactation. The high incidence of pregnancy periods lasting more than 34 days

TEXT-FIG. 1. Daily food consumption of lactating rats on three feeding regimens.

among the restricted females implies that implantation had been delayed after Day 18 of *post-partum* pregnancy, at which time the food restriction was lifted.

None of the lactating females had implantation sites at Day 5 of *post-partum* pregnancy, regardless of feed regimen, but all of them had free blastocysts (Table 2). With decreasing food consumption, fewer females had implantation sites at Day 12 and 18 of *post-partum* pregnancy and more females had free blastocysts (Table 2). The number of fetuses in the dams with implantation

Table 1. Gestation length and prolificacy of lactating rats under three feeding regimens

Feed level	No. of females	No. of parturients*	Pregnancy period* (days)	Mean litter size
Free fed	14	11	24 to 26 (11)†	9.4
62% of free fed	7	4	25 (1) 37 to 38 (3)	8.0 7.6
41% of free fed	13	7	26 (1) 34 to 38 (3) 28 to 30 (3)‡	Dead 10.6 10.8

* The '62%' and '41%' groups differed significantly from the 'free-fed' group in the pregnancy period (χ^2 test), but the difference in the number of parturients was not statistically significant.

† The numbers of animals are in parentheses.

‡ These three females ceased lactating after the 12th to 14th day of pregnancy and the young died.

TEXT-FIG. 2. Growth of young nursed by mated female rats on three feeding regimens.

Table 2. Incidence of blastocysts and implantation sites in rats at different times during *post-partum* pregnancy under three feeding regimens

Feed level	No. of females	Females with fetuses		No. of females with blastocysts	No. of females with empty uteri
		No. of females	No. of fetuses		
Day 5					
Free fed	5	0	—	5	0
41% of free fed	5	0	—	5	0
Day 12					
Free fed	7	6	13.5	0	1
62% of free fed	10	6	11.6	1	3
41% of free fed	7	2	12.5	5	—
Day 18					
Free fed	10	9	10.1	0	1
62% of free fed	8	5	9.6	2	1
41% of free fed	8	2	16.0	6	0

sites in the free-fed and 62% groups was similarly reduced from Day 12 to Day 18 of pregnancy. Thus, in the present study, food restriction did not aggravate the rate of postimplantation losses, which are known to occur during lactation (McCarthy, 1965).

The young gained weight similarly in the three feeding groups until the 5th day of lactation but from then on, their weight gain was proportional to their dams' feed consumption (Text-fig. 2).

DISCUSSION

A low incidence of delay in implantation beyond 7 days and up to 16 days in lactating rats has been described by Mantalenakis & Ketchel (1966), but the possible connection with the energy status of the animal was not discussed by them. The present results show that implantation in lactating rats can be postponed at least until the 18th day, if food restriction is imposed on them.

Although the rats on restricted diets were similar to the control group in the incidence of empty uteri and in the postimplantation losses, they still differed from the controls in the lower delivery rate. As shown, food restriction led to prolonged preservation of the embryos in the stage of free blastocysts, though preservation of blastocysts does not necessarily imply normal implantation and delivery. Considering the precise timing of maturity of the blastocysts and the receptivity of the uterus necessary for implantation (Psychoyos, 1967), it is likely that the lower delivery rate among the restricted females is due mainly to unaccomplished implantation after interruption of the long blastocystic diapause.

Food restriction, apart from prolonging the delay of implantation, depressed the weight gain of the young (Text-fig. 2).

Increased litter size was found to affect the delay in implantation (Mantalenakis & Ketchel, 1966) and the weight gain of the young (Schultze, 1954; Widdowson & McCance, 1960) in a way which resembled that of food restriction, and an association between retarded sucklings and an extended delay of implantation can be assumed. Wiesner & Sheard (1933) suggested that the effectiveness of the maternal responses evoked by the young is inversely related to their age. One might speculate that retarded young, considered to be physiologically younger than their chronological age (Widdowson, 1967), maintain an intensive 'suckling stimulus' which, according to Rothchild (1960), depresses gonadotrophic activity and thus extends the delay of implantation.

This paper supports the suggestion, first proposed by Lataste (1891), that the cause of delay in implantation is the competition of the young for energy supply. We suggest that at the initial stages of heavy lactation, even free food consumption is inadequate for meeting the energy demands of the growing young and the mated females. Under conditions of food restriction, the competition of the young for energy becomes even more critical, leading to a further delay in implantation. Increasing food consumption with advanced lactation tends to overcome the energy drainage due to milk production and to satisfy the sucklings' demands, thus reducing the exposure of the females to the suckling stimulus and to energy deficit, and permitting implantation.

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